

Research Article

Heme Oxygenases-1 (Hmox-1) and Serum Ferritin Level
between Preeclampsia and Normal Pregnancy*Kadar Heme Oksigenase-1 (Hmox-1) dan Ferritin Serum pada
Pasien Preeklamsia dan Kehamilan Normal*

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Abstract

Objective: To determine the comparison of Hmox-1 to serum ferritin level between patients with preeclampsia and normal pregnancy.**Methods:** This study used analytic observational with cross sectional design. We included 30 subjects with preeclampsia and the other 30 people with normal pregnancy in accordance with inclusion and exclusion criteria. Examination of Hmox-1 and ferritin level was performed through ELISA method. The data consisted of physical and laboratory examination and they would be continued to the calculation in the statistical analysis.**Results:** The average of Hmox-1 level in normal pregnancy and preeclampsia was 1.2 (SD 1.6) ng/ml and 0.3 (SD 0.2) ng/ml ($p < 0.05$). The average difference in ferritin level in normal pregnancy and preeclampsia was 32.9 (SD 56.0) ng/ml and 43.0 (SD 45.2) ng/ml ($p < 0.05$). Meanwhile, there was no correlation between Hmox-1 and ferritin serum in normal ($r = 0.131$) and preeclamptic ($r = 0.174$) group ($p > 0.05$).**Conclusion:** The level of Hmox-1 in preeclamptic group is lower than normal pregnancy; while, the ferritin level in women with preeclampsia is higher than normal pregnancy. There is no significant correlation between Hmox-1 and ferritin serum level.

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Keywords: ferritin, Hmox-1, preeclampsia

Abstrak

Tujuan: Untuk menentukan perbandingan kadar Hmox-1 terhadap ferritin serum pada pasien preeklamsia dan kehamilan normal.**Metode:** Penelitian ini adalah analitik observasional dengan rancangan potong lintang. Sebanyak 30 orang masing-masing pada setiap kelompok digunakan sebagai subjek preeklamsi dan subjek kehamilan normal (kontrol) yang sesuai dengan kriteria inklusi dan eksklusi. Kami melakukan pemeriksaan kadar Hmox-1 dan kadar ferritin dengan ELISA Kit serta pemeriksaan fisik dan laboratorium, kemudian dilakukan perhitungan secara statistik.**Hasil:** Dari penelitian, diperoleh rerata kadar Hmox-1 pada kehamilan normal dan preeklamsia adalah 1.2 (SB 1.6) ng/ml dan 0.3 (SB 0.2) ng/ml dengan menunjukkan perbedaan yang bermakna ($p < 0.05$). Rerata kadar ferritin pada kehamilan normal dan preeklamsia adalah 32.9 (SB 56.0) ng/ml dan 43.0 (SB 45.2) ng/ml dengan perbedaan yang bermakna ($p < 0.05$). Sementara itu, hasil korelasi antara kadar Hmox-1 dan ferritin serum untuk kehamilan normal dan preeklamsi adalah $r = 0.131$ dan $r = 0.174$ dengan perbedaan yang tidak bermakna ($p > 0.05$).**Kesimpulan:** Kadar Hmox-1 pada penderita preeklamsia lebih rendah dibandingkan dengan kehamilan normal, sedangkan kadar ferritin pada penderita preeklamsia lebih tinggi dibandingkan dengan kehamilan normal. Kadar Hmox-1 dan ferritin serum tidak berkorelasi secara bermakna.

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Kata kunci: ferritin, Hmox-1, preeklamsia**Correspondence:** Ridwan A Putra, graha2rap@yahoo.com

INTRODUCTION

Preeclampsia is one of pregnancy complication characterized by hypertension (systolic blood pressure more than 140 mmHg or diastolic blood pressure more than 90 mmHg) and proteinuria (≥ 300 mg/24 hours) at the gestational age of more than 20 weeks.¹ Preeclampsia impacts to morbidity and mortality for the mother and fetus. Several effects influencing women include of complication of HELLP syndrome (hemolysis, elevated liver enzymes, low platelet level), pulmonary edema,

kidney disorder, bleeding, placental abruption or even death.^{1,2} Meanwhile, in infant, it can lead to complication, such as premature birth, fetal distress, low birth weight (LBW) or intra uterine fetal death (IUFD).³

Preeclampsia is the leading cause of women's death contributing around 24%, below the bleeding cause as 28%.⁴ Based on data from incidence of preeclampsia in Dr. Hasan Sadikin Hospital Bandung, the occurrence of bleeding was happened between 4.0 and 10.4%; while, the

incidence of preeclampsia was ranged from 2.3 to 4.3% in 2008 to 2010. In 2013, the incidence of preeclampsia in Dr. Hasan Sadikin hospital increased to 13.6% of total patients in obstetrics room with the average number of patients per month were as many as 38 people.⁵⁻⁸

The pathophysiology of preeclampsia is still unknown.⁹ However, placental ischemic factor and angiogenic imbalance are considered as major causes of preeclampsia. Ischemic in placenta is caused by destruction of red blood cells, especially in the area of the placenta so that it results to the release of hemoglobin/heme and Fe in large quantities into the circulation. It can lead to the induction of ferritin system. Ferritin is the most important protein storing iron/Fe.¹⁰

Another factor of this cause is an angiogenic imbalance. According to study by Ahmed, they said that the rising level of soluble Fms-like tyrosine kinase receptor-1 (sFLT-1), zinc (soluble endoglin), and the decreasing level of placental growth factor (PlGF) had a major role for the incidence of preeclampsia. The increase of sFlt-1 caused endothelial damage and inhibited angiogenesis through binding and playing an antagonist role on the function of vascular endothelial growth factor (VEGF) and PlGF, also increasing oxidative stress and inflammation. The increase level of zinc caused limited signaling activity of transforming growth factor β 1 (TGF β 1) and endothelial nitric oxide synthase (eNOS) that influenced to vessel damage and inhibited angiogenesis.⁹

The recovery of angiogenic balance becomes important and it is believed having effective result in the treatment of preeclampsia. Recent study showed pharmacological role of Heme oxygenase-1 (Hmox-1) and the metabolites (biliverdin, free Fe, and carbon monoxide) on preeclampsia by restoring the balance of angiogenic and reducing oxidative stress in the ischemic placenta.^{11,12} Kawashima, et al. in his study stated that there was a relationship among the level of Hmox-1, ferritin, and bilirubin in patients suffering from anemia, growth inhibition, and hyperlipidemia.

Therefore, this study aims to determine the comparison of Hmox-1 to serum ferritin level between patients with preeclampsia and normal pregnancy.

METHODS

Research Subject

The subjects were 60 pregnant women coming to perform antenatal care in Dr. Hasan Sadikin Hospital and network hospitals, from December 1, 2014 to February 28, 2015. The study subjects had to meet the inclusion and exclusion criteria.

Clinical Examination Method

The study carried out a regular examination to determine the diagnosis of preeclampsia, such as blood pressure and proteinuria. The inclusion criteria of normal pregnancy group were single fetus of intrauterine pregnancy, gestational age ≥ 20 weeks from last menstrual period (LMP) or first sonography result, normal blood pressure and negative proteinuria. For preeclampsia group, it included single fetus of intrauterine pregnancy, gestational age ≥ 20 weeks from LMP or first sonography result, systole blood pressure ≥ 140 mmHg and diastole blood pressure 90 mmHg, also positive proteinuria. We excluded women with heart congenital fetus, infection, chronic diseases of women including kidney, heart, hypertension, hypothyroid and diabetic diseases, smoker and alcoholic, intrauterine growth retardation (IUGR). We had performed matching for both groups.

Laboratory Examination Method

We examined the blood and protein level in the Laboratory of Clinical Pathology, Dr. Hasan Sadikin Hospital in accordance with national standard. Blood samples were obtained through phlebotomy conducted by health professional in the Department of Obstetrics and Gynecology, Dr. Hasan Sadikin Hospital on the cubital vein using a disposable 5 ml syringe. We put the blood sample in plain vacutainer and it was taken to the Laboratory of Clinical Immunology Department of Clinical Pathology, Dr. Hasan Sadikin hospital. Blood samples were let standing for around 30 minutes until they were freezing and then centrifuged at 3,000 rpm for 10 minutes. The result of serum was separated and put into plastic tubes and stored at -20°C . Later, examination of Hmox-1 level was performed by using ELISA Kit. Meanwhile, ferritin level was measured by using the principle of Sandwich and analyzed through Ferritin Kit No. 03737551 COBAS.

Data Analysis

Analysis was formerly performed with normality test. For categorical data analysis in both groups, we used the chi-square (χ^2) or the Fisher exact whether it was found the expected value of less than 5. To compare the average value of quantitative data, we analyzed through t-test for normally distributed data and Mann-Whitney test for not normally distributed data. To analyze the correlation between the four independent variables, it applied Pearson correlation analysis. All of them was operated using SPSS for Windows version 21.0.

RESULTS

A total of 60 subjects met the criteria for inclusion, which consisted of 30 pregnant women with preeclampsia and 30 normal pregnant women as a control. We collected the characteristics demographic for both groups starting from blood pressure, Hmox-1, and ferritin level examination. Table 1 depicted the average level of Hmox-1 between normal and preeclamptic group.

Table 1. The Comparison of Hmox-1 Level between Normal and Preeclampsia Women

Hmox-1 Level (ng/ml)	Group		Statistical Test
	Normal (n=30)	Preeclampsia (n=30)	
Average (SD)	1.2 (1.6)	0.3 (0.2)	0.011*
Median	0.4	0.3	
Min-max	0.1-6.2	0.01-1.2	

*Mann-Whitney test

Average result of Hmox-1 level in preeclampsia group showed lower values (0.3 (SD 0.2) ng/ml) compared with normal group ($p=0.011$). Study by Farina, et al. stated that Hmox-1 mRNA level in women with preeclampsia was lower than normal pregnant women.¹³ However, another study said that the average level of Hmox-1 in preeclampsia was higher than normal pregnancy group.^{14,15}

Table 2. The Comparison of Ferritin Level between Normal and Preeclampsia Women

Ferritin Level (ng/ml)	Group		Statistical Test
	Normal (n=30)	Preeclampsia (n=30)	
Average (SD)	32.9 (56.0)	43.0 (45.2)	0.028*
Median	14.4	22.5	
Min-max	4.6-246.1	6.6-214.2	

*Mann-Whitney test

Table 2 showed the result of average ferritin level between normal and preeclampsia group: It pointed out that the average ferritin level in preeclampsia group was higher (43 (SD 45.2) ng/ml) compared with normal pregnancy group (32.9 (SD 56.0) ng/ml). It was different significantly ($p=0.028$). Fatima, et al. study indicated that the average value of serum ferritin in patients with preeclampsia was higher at 100.03 (SD 123.52) $\mu\text{gm/l}$ and the value of control group was at 31.53 (SD 20.86) $\mu\text{gm/l}$ ($p<0.001$).¹⁰ Some studies also showed similar result to Taheripanth and Farkush,¹⁶ Zafar and Iqbal,¹⁷ and Siddiqui, et al.¹⁸

Table 3. The Correlation of Hmox-1 and Ferritin Level between Normal and Preeclampsia Group

Correlation	Group	
	Normal (n=30)	Preeclampsia (n=30)
Hm0x-1 and ferritin level	$r=-0.131$ $p = 0.494$	$r=0.174$ $p = 0.357$

r = pearson correlation coefficient

Based on Pearson correlation analysis, there was not correlation between Hmox-1 and ferritin level both in normal and preeclampsia group ($p>0.05$) and the power of correlation was poor ($r=0.131$ and $r=0.174$) (Table 3).

DISCUSSION

The result showed that the level of Hmox-1 in patients with preeclampsia was lower than normal pregnancy. Meanwhile, ferritin level in patients with preeclampsia was higher than normal pregnancy. High ferritin level indicated that there had been an increasing on the induction of ferritin system. The increasing induction of ferritin system

was caused by placental ischemia that occurred in patients with preeclampsia. In addition, the patients with preeclampsia, it was also characterized by an imbalance of angiogenic factors in their body that was indicated by the decreasing level of Hmox-1. Therefore, the result of this study described that patients with preeclampsia had low level of Hmox-1 and high ferritin level.

During pregnancy, serum ferritin level would reach the lowest point up to 20 ng/dl in the third trimester of pregnancy. An individual who had a serum ferritin value >400 mg/l could be said that these individuals experienced abnormalities of iron related to tumor. While, the raising of serum ferritin in pregnancy was influenced by hypertension and eclampsia.

There was no correlation between Hmox-1 and ferritin level in both preeclampsia and normal pregnant women. Temporary assumption was that higher ferritin level might be caused by ischemic placental. This condition was suffered from patients with preeclampsia.

CONCLUSION

The level of Hmox-1 in preeclamptic group is lower than normal pregnancy; while, the ferritin level in women with preeclampsia is higher than normal pregnancy. There is no significant correlation between Hmox-1 and ferritin serum level.

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